ABSTRACT OF THE DISCLOSURE

Detection of variable nucleotide(s) is based on primer extension and incorporation of detectable nucleoside triphosphates. By selecting the detection step primers from the region immediately adjacent to the variable nucleotide, this variation can be detected after incorporation of as few as one nucleoside triphosphate. Labelled nucleoside triphosphates matching the variable nucleotide are added and the incorporation of a label into the detection step primer is measured. The selection of the detection step primer is important to the method according to this invention and is dependent on the nucleotide sequence of interest. The detection step primers are preferably selected so as to span the region immediately toward the 3' end from the variable nucleotide to be detected. The detection step primers can also be complementary to a sequence beginning several nucleotides removed from the variable nucleotide. The only limitation concerning the position of the detection step primers is that the sequence between the 3' end of the detection step primer and the variable nucleotide to be detected must not contain a nucleotide residue of the same type as the one to be detected. The detection step primers can equally well be chosen to be complementary to either the coding or non-coding strands of the nucleotide sequence of interest.